The Structure and Hybridization Properties of DNA Monolayers Engineered for Complex Media Applications

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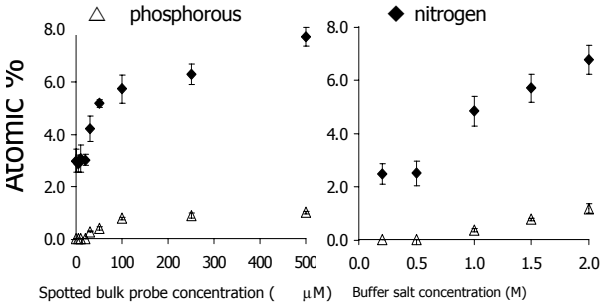
Statement of Purpose: Reliable, direct capture of nucleic acid targets from complex media would greatly improve existing capabilities of DNA microarrays and biosensors. This goal remains a challenge for many current nucleic acid detection technologies attempting to produce assay results directly from complex real-world samples. In this study, we have investigated the surface structure and performance of thiolated single-strand DNA (HS-ssDNA) attached to a maleimide-ethylene glycol disulfide (MEG) monolayer on gold. These results are compared to previous results published for HS-ssDNA monolayers on gold backfilled with either 11-mercapto-1-undecanol (MCU) and 11-mercapto-undecyl tetra ethylene glycol (OEG).[1-3] The attachment chemistry and surface coverage of ssDNA probes were studied by x-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS). ssDNA probe orientation was determined by near edge x-ray absorption fine structure (NEXAFS). Target DNA hybridization on DNA probe surfaces was monitored by surface plasmon resonance (SPR). Hybridization from buffer and serum was examined to determine the amount and effects of non-specific adsorption on DNA hybridization.

Methods: For XPS and NEXAFS experiments Si wafers were coated with 10nm Ti and 100nm Au. For SPR experiments glass slides were coated with 2nm Cr and 50nm Au. The DNA probe surfaces were prepared by first assembling a monolayer of MEG (Prochimia) onto Au from 0.1 mM ethanol solutions. Then after an ethanol rinse the samples were spotted with 5 to 500 µM HS-ssDNA (TriLink Biotechnologies) in 0.2 to 2 M SSC buffer (1.5 M NaCl, 0.015 M Na citrate, pH 7) and incubated at RT under 100% humidity for 1.5 h. ssDNA probe density was calculated from the XPS P concentration using a ^{32}P calibration curve.[1,2] Negative controls included exposing (1) MEG monolayers to non-thiolated DNA and (2) MEG monolayers pre-treated with 0.5 mM mercapto-1-hexanol (Sigma-Aldrich) to HS-ssDNA. The XPS compositional and high-resolution spectra were acquired on a Kratos AXIS Ultra DLD spectrometer with a monochromatic Al-Kα X-ray source. ToF-SIMS positive and negative secondary ion spectra were acquired with a PHI Model 7200 instrument using a Cs^+ primary ion source. The NEXAFS spectra were acquired on beamline U7A at Brookhaven National Laboratory. A Plasmon-II SPR instrument with a dual channel flow cell was used to measure DNA hybridization from STE buffer (1M NaCl, 10 mM Tris-HCl, 1 mM EDTA, pH 7.4) and fetal bovine serum (Cambrex).

Results/Discussion: The XPS determined MEG monolayer composition (77 at% C, 18 at% O, 3 at% N and 1 at% S), with the exception of S, was within experimental error of the expected stoichiometry of the MEG molecule. The lower XPS S concentration was due to its location at the MEG/Au interface and attenuation by the MEG monolayer. Angle-dependent XPS showed the maleimide and ethylene glycol portions of the MEG molecules were located at the outer surface of the monolayer. When the MEG monolayer was exposed to HS-ssDNA, P was detected and the N concentration increased, both consistent with the attachment of HS-ssDNA to the MEG surface. The amount of P and N detected depended on the HS-ssDNA solution concentration and the buffer ionic strength (see Figure 1). ToF-SIMS results also showed that HS-ssDNA was attached to the maleimide group via the thiol group. NEXAFS showed slight orientation of the ssDNA at low probe densities (~$2x10^{12}$ molecules/cm^2), but showed upright orientation of the ssDNA at high probe densities (>10^{13} molecules/cm^2). No ssDNA attachment to MEG surfaces was detected in the negative control experiments. Hybridization efficiency, as measured by SPR, varied from 90% (low probe densities) to 15% (high probe densities). No binding of non-complementary DNA was detected by SPR. The DNA/MEG system was also more effective than the previous DNA/MCU and DNA/OEG systems for inhibiting non-specific adsorption of protein from serum solutions.

Figure 1. XPS N and P concentrations for HS-ssDNA immobilization onto MEG monolayers as a function of HS-ssDNA concentration (left) and ionic strength (right).



Conclusions: HS-ssDNA probes were successfully immobilized onto a MEG monolayer at densities from $2x10^{12}$ to $3x10^{13}$ molecules/cm^2. The target hybridization efficiency varied from 90 (low density) to 15% (high density). The amount of non-specific adsorption of proteins, etc. from serum decreased as DNA/MCU > DNA/OEG > DNA/MEG.

References:

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